

Appl. No. 09/700,712  
Amendment dated: January 24, 2005  
Reply to OA of: September 23, 2004

### REMARKS

Applicant has amended the specification and claims to more particularly define the invention taking into consideration the outstanding Official Action. The specification at page 4 has been amended to indicate that sequence No. 1 shown on Figure 1 and Fig. 1 (cont) is shown as Fig. 1(a) and Fig. 1(b) in accordance with the Examiner's requirement. The specification has been reviewed with respect to the use of Trademarks and will be appropriately amended when there is an indication of allowable subject matter as agreed with Examiner Devi during a telephone conversation of January 24, 2005. Perkin Elmer and Pharmacia are well known companies in the field as is Promega.

All of the claims in the application have been canceled without prejudice or disclaimer. New claims 17-23 have been added. Claim 17 is a combination of claims 3 and 7, claim 18 is based upon claim 1 and claims 4-6 are now 19-21 and previous claim 8 is now claim 22 and previous claim 9 is now claim 23. Applicant most respectfully submits that all the claims now present in the application are in full compliance with 35 U.S.C. §112 and are clearly patentable over the references of record.

The rejection of claims 2, 9 and 12 under 35 U.S.C. 101 as being directed to a non-statutory subject matter has been carefully considered but is most respectfully traversed in view of the cancellation of these claims from the application. Accordingly, it is most respectfully requested that this rejection be withdrawn.

Applicants do not agree with the Examiner that claim 2 does not sufficiently distinguish over naturally occurring spontaneous mutants of *Vibrio cholerae* lacking the functionality of the *thyA* gene. In the PCT application, first page, lines 4, 5 is disclosed that the invention relates to a strain of *Vibrio cholerae* that has been deprived of its *thyA* gene in the chromosome, i.e. a  $\Delta$  *thyA* strain lacking the functionality of the *thyA* gene. Thus, Applicants are of the opinion that the " $\Delta$ " indicates that a man has made the strain

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that lacks the functionality of the *thyA* gene. Applicants enclose a page 269 from the New England BioLabs Inc. 2005.05 Catalog & Technical References form which it is evident that a  $\Delta$  mutation present is known to be a large deletion.

However, Applicants have decided to concentrate this application to  $\Delta$  *thyA* mutants of *V. cholerae* that can be used as suitable production strains for recombinant proteins encoded on plasmids. (See PCT application page 1, lines 6-10, page 3, lines 2-6 and page 5, lines 31, 32.)

On top of page 5 of the Official Action the Examiner has objected to the expression "gene... having essentially the nucleotide sequence SEQ ID NO. 1". Therefore, in the new claim 17, Applicants have inserted instead the expression used for "having essentially the nucleotide sequence" from PCT text page 3, lines 9-14.

Applicants have inserted "the" instead of "a" in the beginning of all the dependent claims.

In the previous claim 8, new claim 22, applicants have inserted "heterologous" between "the encoded" and "protein".

Since Applicants no longer claim a nucleotide sequence of a *thyA* gene of *Vibrio cholerae* as such as no vaccine, the objections of items 15-17 are no longer applicable. Applicants most respectfully submit that one of ordinary skill in the art would appreciate that the claims are not drawn to products of nature. In this regard, the Examiner's attention is directed to United States Patent 6,723,323, issued by Examiner which cites the corresponding WIPO publication of the present application as prior art. This United States patent is representative of the state of the level of skill of one in the prior art to which the invention pertains. This level of skill is pertinent to evaluating the patentability of the presently claimed invention. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 1-9, 12, 13 and 16 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the

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subject matter which applicants regard as the invention has been carefully considered but is most respectfully traversed in view of the cancellation of the above claims and the presentation of a new claim set. In rewriting the claims it is believed that the various rejections under 35 USC 112 have been obviated. Accordingly, it is most respectfully requested that this rejection be withdrawn.

The rejection of claims 9 and 12 under 35 U.S.C. §102(b) as being anticipated by Valle et al.; claims 2 and 16 as being anticipated by Attridge et al. and Mahan et al.; and claims 2-7 by Morona et al. has been carefully considered but is most respectfully traversed in view of the cancellation of these claims. It is therefore believed that this rejection has been obviated and it is most respectfully requested that these rejections be withdrawn.

The rejection of claim 8 under 35 U.S.C. 103 as unpatentable over Morona et al. in view of Arntzen et al.; claims 1 and 16 as being unpatentable over Valle et al. in view of Morona et al. and claim 13 over Valle et al. in view of Morona et al. has been carefully considered but is most respectfully traversed.


The Examiner cites Morona et al. that disclose a mutant strain of *Vibrio cholerae* defective (i.e. lacking functionality) in the *thyA*<sup>+</sup> gene. However, the Examiner is not correct in stating that the mutant strain grows on a media containing no thymine, since by definition a *thyA* strain does not grow on media containing no thymine. Morona et al. discloses selection of mutant strains, i.e. point mutations in the strain and not defined *thyA* mutants of *Vibrio cholerae* as in the present case. As already discussed in the PCT application, page 2, lines 6-16, the strains of Morona et al. may revert to wild-type at unacceptable high frequencies. All of the claims have been cancelled without prejudice or disclaimer. It is therefore believed that these rejections have been obviated. Accordingly, it is most respectfully requested that these rejections be withdrawn.

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In view of the above comments and further amendments to the specification and claims, favorable reconsideration and allowance of all of the claims now present in the application are most respectfully requested.

Respectfully submitted,

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**Amendments to the Drawings:**

Please replace the original drawing FIG. 1 and FIG. 1(cont.) on pages 1/18 and 2/18 with the following amended drawings FIG. 1(a) and FIG. 1(b) found at the end of this paper and marked "Replacement Page".

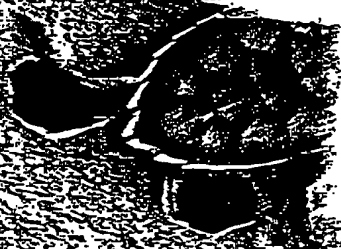
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NEW ENGLAND  
**Biolabs** inc.

2005-06 Catalog  
& Technical Reference

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## Strain Phenotype: McrA, McrBC, EcoK

Strain	Mcr A	Mcr BC	R	EcoK M	Strain Background	Strain	Mcr A	Mcr BC	R	EcoK M	Strain Background
1100	+	+	+	+		K802	see WA802				
AB265	+	+	+	+		K803	see WA803				
AB1157	+	+	+	+	Mrr	K10	+	+	+	+	Hlr Cavalli
AT2459	+	+	+	+		KMBL1154	+	?	+	+	
BNN93	+	+	+	+	C600	KW251*	+	+	+	+	
BNN102	+	+	+	+	C600	LE392	+	+	+	+	COB-54
CH731*	+	?	?	?		MB406*	+	+	+	+	
CH1332*	+	?	?	?	CH731	MC1040	(+)	(+)	+	+	
CH1372*	+	?	?	?	CH731	MC1061	+	+	+	+	Mrr
C235	+	+	+	+		NM294	+	+	+	+	1100, Mrr
C600	+	+	+	+	Y70, Mrr	NM477	+	+	+	+	C600
CES200	+	+	+	+	AB1157	NM494	+	+	+	+	pop'01
CJ236** (E4115)	+	+	+	+		NM514	+	+	+	+	pop'01
CPB1293	+	+	+	+	W3110	NM519	+	+	+	+	
CPB1321	+	+	+	+	W3110	NM538	(-)	(+)	+	+	ED8554
CR63	+	+	+	+		NM539	+	+	+	+	NM538
CSR603	+	+	+	+		NM554	+	+	+	+	MC1061
X2813	+	+	+	+	WA802, Mrr	NM621	+	+	+	+	C600
DB1316*	+	+	+	+	Mrr	NM646*	+	+	+	+	WA102
DH1	+	+	+	+	LM294, Mrr	NM664*	+	+	+	+	WA402, Mrr
DH3	+	+	+	+	MM294, Mrr	NM670*	+	+	+	+	WA102
DH5	+	+	+	+	LM294, Mrr	NM673*	+	+	+	+	WA102
DH5αMCR*	+	+	+	+	DH5, Mrr	NM674*	+	+	+	+	WA402, Mrr
DH10B*	+	+	+	+	MC1061, Mrr	NW1*	+	+	+	+	AT2459
DL538*	+	+	+	+	NM621	NW2*	+	+	+	+	AT2459, Mrr
DS410*	+	+	+	+		P678**	+	+	+	+	
DM800	+	+	+	+		PA309	+	+	+	+	
ED8641	+	+	+	+	WA803	PC0543**	+	+	+	+	Mrr
ED8654	+	+	+	+	WA803	PC0950	+	+	+	+	Mrr
ED8739	+	+	+	+	WA803	Q358	+	+	+	+	
ED8767	+	+	+	+	WA803	Q359	+	+	+	+	
ER1370	+	+	+	+	NK7254 (JC1552)	RL88	+	+	+	+	
ER1381	+	+	+	+	ER1370, Mrr	RR1*	+	+	+	+	HB101, Mrr
ER1378	+	+	+	+	ER1370, Mrr	SKS022	+	+	+	+	
ER1398	+	+	+	+	MM294, Mrr	TC410*	+	+	+	+	DS410, Mrr
ER1414	+	+	+	+	W3110, Mrr	TC600*	+	+	+	+	C600
ER1458	+	+	+	+	Y1084	W5	+	+	+	+	
ER1562	+	+	+	+	MM294, Mrr	W677**	+	+	+	+	
CR1563	+	+	+	+	MM294, Mrr	W3110	+	+	+	+	Mrr
ER1564	+	+	+	+	ER1370, Mrr	W4797	+	+	+	+	
ER1565	+	+	+	+	ER1370, Mrr	WA704	+	+	+	+	C600 x HlrCavalli
ER1647*	+	+	+	+	ER1370, Mrr	WA802	+	+	+	+	WA704, Mrr
ER1648	+	+	+	+	ER1370, Mrr	WA803	+	+	+	+	WA704, Mrr
ER1793** (E41015)	+	+	+	+	ER1370, Mrr	WW3352	+	+	+	+	
ER1821** (E41025)	+	+	+	+	MM294, Mrr	X149	+	+	+	+	
ER2267** (E41035)	+	+	+	+	MM294, F, Mrr	Y10	+	+	+	+	Y10
ER2095 (E41095)	+	+	+	+	GM271, Mrr	Y53	+	+	+	+	Y53
ES1505	+	+	+	+	C600	Y70	+	+	+	+	BRN98, SG1041
GM31*	+	+	+	+	Mrr	Y1084	+	+	+	+	LE392, ED8554
GM161	?	+	+	+		Y1088	+	+	+	+	Y1084
GM271	?	+	+	+	Mrr	Y1090	(-)	(+)	+	+	
GM272	?	+	+	+							
GM2159**	+	+	+	+	Mrr						
GM2929**	+	+	+	+	GM2163 recF						
GW1002	+	+	+	+							
H680	+	+	+	+							
H6101*	+	+	+	+	Mrr						
Hlr3000 YA149	+	+	+	+	HlrH						
Hlr3000	+	+	+	+							
Hlr Cavalli	+	+	+	+							
Hlr Hayes**	+	+	+	+	Mrr						
HlrH mi*	+	+	+	+							
HlrP4X6	+	+	+	+							
HlrR4**	+	+	+	+	Mrr						
HL1228*	+	+	+	+	NM621						
JC7623**	+	+	+	+	Mrr						
JM132*	+	+	+	+	K802						
JM33	(+)	+	+	+							
JM101	+	+	+	+	JM83						
JM107	+	+	+	+	MM294, Mrr						
JM107MA2	+	+	+	+	JM107						
JM109	(-)	(-)	+	+	JM107						
5K	+	+	+	+	C600						
K12	+	+	+	+							

## Non-K12 strains

E. coli B

E. coli B/r

BL21\*\*

E. coli C

Mcr phenotypes reported in reference 7 except those denoted with asterisks. Mrr phenotypes determined at NEB except DH105 (11). EcoK1 phenotype; sometimes inferred from genotype.

(-) phenotype inferred from that of ancestral or descendant strains.

7 phenotype not tested.

Δ mutation present is known to be a large deletion.

† These strains were formerly thought to carry the *mcrB* allele present in *E. coli* B. They are now known to carry an extensive deletion of *mrr* + *hsd* + *mcrBC* (ref. 6; M. Noyer-Wedner, personal communication; J. Kelleher, personal communication).

- Phenotypes reported in one of references 8-12.

\*\* Phenotypes determined at New England Biolabs, Inc.

†† Catalog numbers indicate strains that are available upon request from New England Biolabs at no charge with an order or for the cost of shipping if ordered separately.

\*\*\* C600 lines obtained from different sources give different results. The original strain, and that obtained from the *E. coli* Genetic Stock Center (Yale University) are *McrA*; derivatives traceable to the Branner laboratory are *McrA* (21).